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14. ABSTRACT

Clinical studies suggest that obesity increases the risk for breast cancer and there is convincing evidence that post-menopausal breast cancer risk is highly correlated with serum estrogen levels. One potential link between obesity and breast cancer risk is increased estrogen production by the adipose tissue itself. The adipose tissue produces the enzyme aromatase which catalyses the biosynthesis of estrogen from androgen and also 17-beta-hydroxysteroid dehydrogenase (17-beta HSD) important for the conversion of estrone to estradiol. Our studies have identified two key molecules (insulin and leptin) in obesity that regulates aromatase and 17-beta HSD synthesis in adipose tissues and in adipocytes. The identification of these target molecules that may ultimately induce estrogen production in the setting of obesity may provide a unique therapeutic preventive strategy to reduce systemic estrogen levels and thereby reduce post-menopausal breast cancer risk associated with obesity.

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Introduction:

Clinical studies suggest and obesity increases the risk for breast cancer and there is convincing evidence that post-menopausal breast cancer risk is highly correlated with serum estrogen levels. One potential link between obesity and breast cancer risk is increased estrogen production by the adipose tissue itself. The adipose tissue produces the enzyme aromatase which catalyses the biosynthesis of estrogen from androgen and also 17-β-hydroxysteroid dehydrogenase (17-βHSD) important for the conversion of estrone to estradiol. In spite of this the mechanisms regulating the adipose expression of aromatase and 17-βHSD is however currently unknown. Identifying the mediators in obesity that regulate aromatase and 17-βHSD synthesis in adipose tissues and in adipocytes may provide a unique therapeutic preventive strategy to reduce systemic estrogen levels and thereby reduce post-menopausal breast cancer risk associated with obesity.

BODY:

Task 1 : Perform in vitro studies on the regulation of Aromatase and 17-βHSD (types 4, 5) synthesis in murine and human adipocytes in response to insulin and leptin

In order to determine the regulation of Aromatase and 17-βHSD in adipocytes we initially standardized an in vitro murine adipocyte cell culture system, In this model, 3T3, L1 pre adipocytes were grown and differentiated into adipocytes after a brief exposure of confluent pre-adipocytes to insulin and dexamathasone. This treatment triggered the differentiation of pre adipocytes to fully differentiated lipid filled adipocytes over the course of 2-3 weeks. Fully differentiated 3T3-L1 adipocytes were treated with insulin (100nM), and cells harvested at various times after treatment for total RNA extraction. Aromatase and 17-βHSD5 mRNA expression was determined by real time RT-PCR. Treatment of fully differentiated 3T3-L1 adipocytes with 100nM
insulin in serum containing media (SCM) significantly induced both Aromatase (Fig 1 A) and 17-βHSD5 mRNA (Fig 1 B) expression in these cells. Aromatase mRNA expression was induced significantly as early as 1 hour after insulin treatment and this expression continued to increase by 3 h after insulin treatment. Similar kinetics of induction of mRNA was also observed after insulin treatment for the expression of 17-βHSD5 mRNA (Fig. 1B). 17-βHSD5 mRNA expression was dramatically induced at 1 and 3 h after insulin treatment. These studies suggest that hyperinsulinemia associated with obesity may contribute to the increased expression of Aromatase and 17-βHSD5 mRNA from adipocytes.

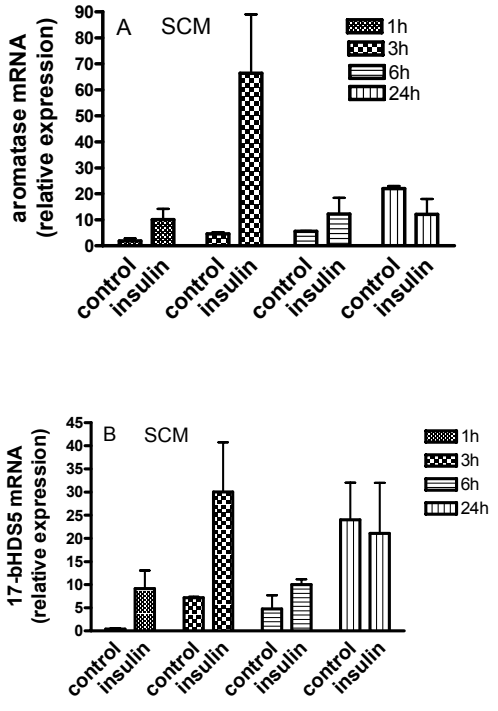

Fig. 1: Aromatase and 17-β HSD5 mRNA expression in response to insulin in cultured 3T3-L1 adipocytes. For all conditions n=6±SD

In a different set of experiments fully differentiated 3T3-L1 adipocytes were treated with leptin (100nM) and the kinetics of both Aromatase and 17-βHSD5 mRNA regulation determined at 3, 6, and 24 hrs after leptin treatment. Aromatase mRNA expression was significantly induced in adipocytes at 3 and 6 hrs after leptin treatment (Fig. 2A). In contrast to Aromatase mRNA expression, the gene expression of βHSD5 was reduced 3 hrs after leptin treatment but was significantly increased after exposure to leptin for 24 hrs. These data suggest that long term chronic exposure to leptin may increase the expression of βHSD5 from adipocytes.

Since human obesity is associated with elevated levels of leptin, our data suggest that hyperleptinemia associated with obesity may contribute not only to Aromatase gene expression but also to increased levels of βHSD5 in adipocytes. These results thus support our primary hypothesis that the hyperleptinemia and hyperinsulinemia associated with obesity may induce the expression of Aromatase and βHSD5 from the adipose tissue, specifically from adipocytes. Studies to determine whether the increase in Aromatase and βHSD5 gene expression in response to insulin and leptin actually leads to increased estrogen secretion into the conditioned media are ongoing.

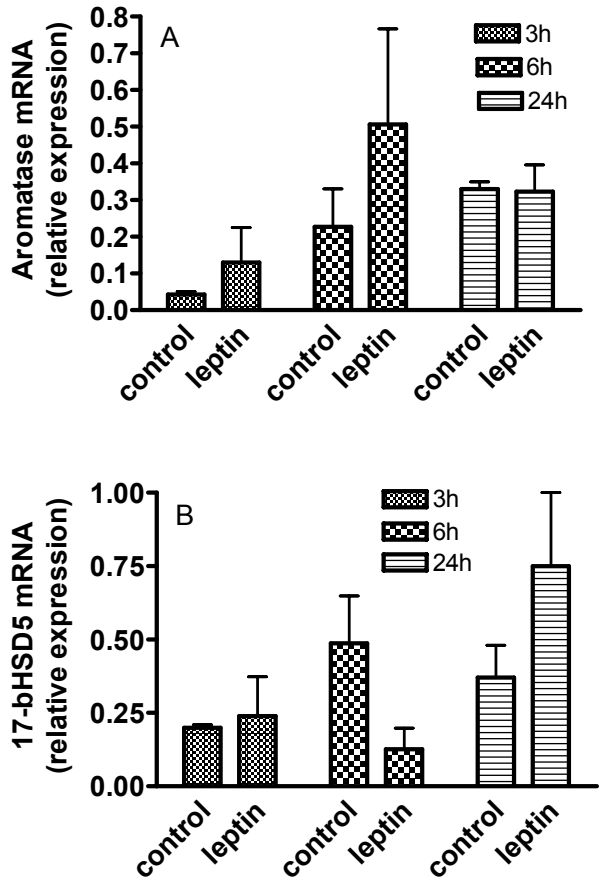

Fig. 2: Aromatase and 17-β HSD5 mRNA expression in response to leptin in cultured 3T3-L1 adipocytes. For all conditions n=6±SD

Since obesity is usually associated with insulin resistance we had also proposed to determine the induction of Aromatase and βHSD5 mRNA expression in response to insulin and leptin in insulin resistant adipocytes. 3T3-L1 adipocytes were treated with low doses (2ng/ml) of tumor necrosis factor α consecutively for 3 days. Metabolic insulin resistance was determined by measuring insulin mediated glucose uptake. Glucose uptake was reduced by 50-70% in adipocytes treated with TNF-α compared to untreated cells, suggesting that glucose uptake was blunted in these cells. Thus, we have been able to standardize conditions in our cell culture system to mimic metabolic insulin resistance. These "insulin-resistant" adipocytes are currently being used to determine insulin and leptin mediated regulation of Aromatase and βHSD5 expression by these cells.

Task 2: Perform in vivo studies on the regulation of Aromatase and 17-beta HSD (types 4, 5) expression in response to leptin and/or insulin using lean, diet-induced obese and genetically obese mice.

Experiments were performed to determine whether insulin induces the expression of Aromatase and 17-βHSD expression in adipose tissues in vivo. Groups (n=6) of C57BL/6J lean mice were injected with insulin (humulin, 5 IU), and 3 hours later, mice were sacrificed and blood and adipose tissues harvested. Total RNA was extracted from adipose tissues and the expression of Aromatase and 17-βHSD mRNA expression determined by real time RT-PCR. As shown in Fig 3, insulin treatment induced a dramatic and significant expression of aromatase mRNA in adipose tissue of lean mice. Our preliminary studies had previously shown that Aromatase gene expression is also induced by insulin in the obese, ob/ob mice that are insulin resistant. Studies are ongoing to confirm and extend the results relating to the insulin induction of aromatase gene expression in adipose tissues from insulin resistant genetic and diet-induced obese mice.

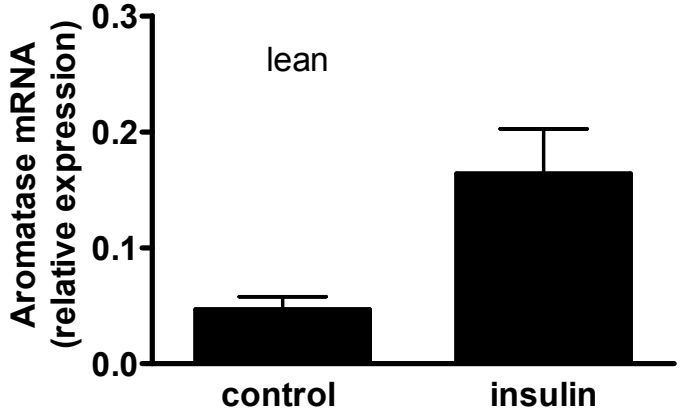

Fig. 3: Aromatase mRNA expression in adipose tissues From insulin treated C57/BL6 lean mice. N=6±SD

We next determined the expression of 17-βHSD mRNA in adipose tissues of insulin treated C57BL/6J lean mice. Interestingly, the expression of 17-βHSD5 mRNA levels was very low in the adipose tissues of these mice and hence we were unable to make any meaningful conclusions in relation to its expression. However, we show that 17-βHSD4 mRNA was expressed in adipose tissues of control untreated C57BL.6J mice, and its expression was significantly and dramatically induced 3 hr after insulin treatment (Fig. 4). These data suggest that the hyperinsulinemia associated with insulin resistance and obesity may drive the expression of both Aromatase and 17-βHSD mRNA and thereby contribute to increased estrogen secretion from adipose tissues in obesity. Studies are ongoing to confirm and extend the results relating to the insulin induction of 17-βHSD gene expression in adipose tissues from insulin resistant genetic and diet-induced obese mice.

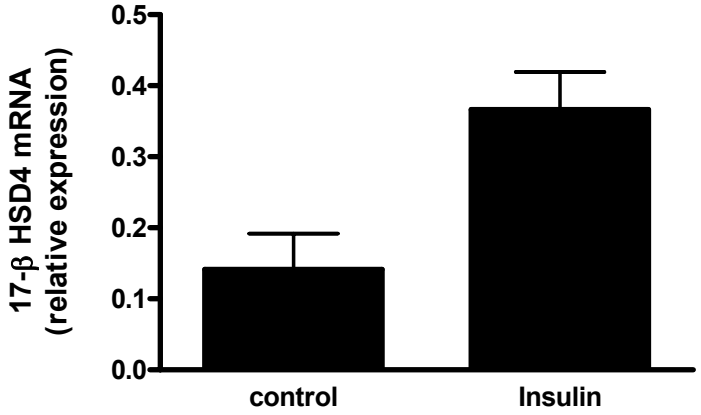

Fig. 4: 17-βHSD mRNA expression in adipose tissues from insulin treated C57/BL6 lean mice. N=6±SD

Experiments were also performed to determine whether the hyperleptinemia associated with obesity also contributes to adipose expression of Aromatase and 17-βHSD mRNA. For these experiments, groups (n=6) of C57BL/6J lean mice were injected with leptin (10µg/mouse). 3 hours later, mice were sacrificed and blood and adipose tissues were harvested. Total RNA was extracted from adipose tissues and the expression of Aromatase (Fig. 5) and 17-βHSD4 mRNA (Fig. 6) expression determined by real time RT-PCR.
As indicated, both Aromatase and 17-βHSD4 mRNA expression was induced significantly in response to leptin in adipose tissues of C57/BL 6J lean mice.

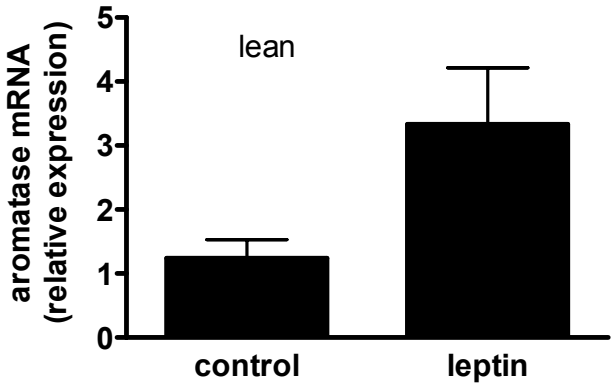

Fig 5: Aromatase mRNA expression in adipose tissues from insulin treated C57/BL6 lean mice. N=6±SD

Together, our in vivo data suggest that increased elevated levels of insulin and leptin, associated with obesity may increase breast cancer risk by inducing the adipose expression of both Aromatase and 17-βHSD. While Aromatase catalyses the biosynthesis of estrogens from androgens, 17-βHSDs are important for the conversion of estrone to estradiol (1). Studies are ongoing to confirm and extend the results relating to the leptin induction of aromatase and 17-βHSD expression in adipose tissues from insulin resistant genetic and diet-induced obese mice.

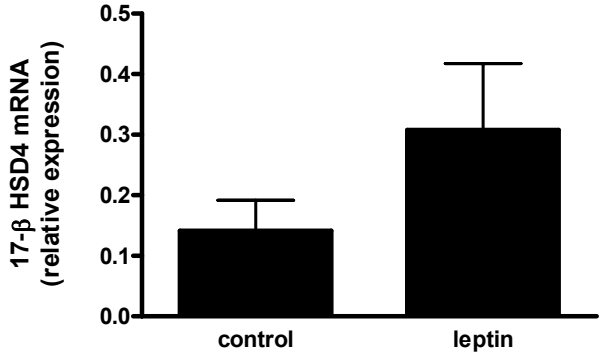

Fig 6: 17-βHSD mRNA expression in adipose tissues from insulin treated C57/BL6 lean mice. N=6±SD

We are currently also in the process of performing insitu hybridization and immunohistochemistry to determine the cellular localization of aromatase and 17-βHSD expression in adipose tissues in response to either insulin or leptin. Estrogen levels in the plasma in response to insulin or insulin also needs to be determined.

Key research Accomplishments:

- Aromatase and 17-β HSD5 mRNA expression is increased in response to **insulin** in cultured 3T3-L1 adipocytes.
- Aromatase and 17-β HSD5 mRNA expression is increased in response to **leptin** in cultured 3T3-L1 adipocytes.
- Aromatase and 17-βHSD4 gene expression is increased in vivo in adipose tissues of C57BL/6J lean mice treated with **insulin**
- Aromatase and 17-βHSD4 gene expression is increased in vivo in adipose tissues of C57BL/6J lean mice treated with **leptin**

Our results thus far have identified two key mediators in obesity (insulin and leptin) that regulates aromatase and 17-βHSD synthesis in the adipose tissue and in adipocytes. The results from this study may provide a unique therapeutic prevention strategy to reduce systemic estrogen levels and thereby reduce postmenopausal breast cancer risk associated with obesity.

Reportable outcomes: None so far

Conclusion:

In conclusion, our studies support the hypothesis that hyperleptinemia and hyperinsulinemia associated with obesity can induce not only aromatase but also 17-β HSD synthesis from the adipose tissue which may lead to an increase secretion of estrogen from an expanded adipose tissue in obesity.

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